

5     **THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY  
OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:**

1.     A method for detecting the presence or absence of a variant nucleotide in at least two  
SNP sites associated with thrombosis, said SNP sites selected from the group consisting of  
10   Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR  
A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T, the method  
comprising the steps of;
- a)     amplifying regions of DNA containing the at least two SNP sites to form  
amplified DNA products;
  - 15     b)     hybridizing at least two tagged allele specific extension primers to a  
complementary target sequence in the amplified DNA products, wherein each tagged allele  
specific extension primer has a 3'-end hybridizing portion substantially complementary to an  
allele of one of the SNP sites associated with thrombosis and a 5'-end tag portion complementary  
to one of a set probes, the terminal nucleotide of the 3' end hybridizing portion being either  
20   complementary to a suspected variant nucleotide or to the corresponding wild type nucleotide of  
the SNP site;
  - c)     extending the at least two tagged allele specific extension primers, using labelled  
nucleotides, if the terminal nucleotide of the 3' end hybridizing portion is a perfect match to an  
allele of one of the SNP sites in the amplified DNA products;
  - 25     d)     hybridizing the at least two allele two tagged allele specific extension primers to  
the set of probes and detecting the presence of labelled extension products.
2.     The method of claim 1 wherein the 3' end hybridizing portions of the at least two tagged  
allele specific extension primers comprise a sequence corresponding to bases 25 and up of any  
30   two of SEQ ID NO: 1 to SEQ ID NO: 12.
3.     The method of claim 2 wherein the 5'-end tag portions of the at least two tagged allele  
specific primers comprises a sequence corresponding to bases 1 to 24 of any two of SEQ ID NO:  
1 to SEQ ID NO: 12.

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- 5     4.     The method of claim 1 wherein the at least two tagged allele-specific extension primers are selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 12.
5.     The method of claim 1 wherein the amplifying step is conducted by PCR.
- 10    6.     The method of claim 1 wherein the probes are coupled to a solid support.
7.     The method of claim 6 wherein the solid support is selected from the group consisting of beads, spectrally coded beads, and a chip based microarray.
- 15    8.     The method of claim 5 wherein the step of PCR amplifying is conducted using a set of PCR amplification primers, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:  
SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and  
SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and  
20    SEQ ID NO: 23 and SEQ ID NO: 24.
9.     A kit for use in detecting the presence or absence of a variant nucleotide in at least two SNP sites associated with thrombosis, said SNP sites selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR  
25    A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T, said kit comprising a set of at least two tagged allele specific extension primers wherein each tagged allele specific extension primer has a 3'-end hybridizing portion substantially complementary to a first allele of one of the SNP sites associated with thrombosis and a 5'-end tag portion complementary to one of a set probes.
- 30    10.    The kit of claim 9 wherein the 3' end hybridizing portion of the at least two tagged allele specific extension primers comprises a sequence corresponding to bases 25 and up of any two of SEQ ID NO: 1 to SEQ ID NO: 12.
11.    The kit of claim 9 wherein the 5'-end tag portion of the at least two tagged allele specific primers comprises a sequence corresponding to bases 1 to 24 of any two of SEQ ID NO: 1 to  
35    SEQ ID NO: 12.

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12. The kit of claim 9 wherein the at least two tagged allele-specific extension primers are selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 12.

10 13. The kit of claim 9 further comprising a set of PCR amplification primers for amplifying regions of DNA containing the at least two SNP sites, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:  
SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24.

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14. The kit of claim 9 further comprising a set of probes.

15. The kit of claim 14 wherein the set of probes are coupled to a support.

20 16. A kit for use in detecting the presence or absence of a variant nucleotide in at least two SNP sites associated with thrombosis, said SNP sites selected from the group consisting of Factor V Leiden G1691A, Prothrombin (Factor II) G20210A, MTHFR C677T, MTHFR A1298C, Factor XIII G4377T, and tissue factor plasma inhibitor (TFPI) C536T, said kit comprising a set of PCR amplification primers for amplifying regions of DNA containing the at  
25 least two SNP sites, said set comprising at least two pairs of PCR primers selected from the group of pairs consisting of:  
SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, and SEQ ID NO: 23 and SEQ ID NO: 24.

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17. The kit of claim 16 further comprising a set of at least two tagged allele specific extension primers wherein each tagged allele specific extension primer has a 3'-end hybridizing portion substantially complementary to a first allele of one of the SNP sites associated with thrombosis and a 5'-end tag portion complementary to one of a set probes.

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